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Grant Number DAMD17-94-J-4265

TITLE: Active Follow-Up of Participants of a Population-Based Specimen Bank (Plasma, DNA, RBC)

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REPORT DATE: October 1997

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for public release;
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19980226 033

DTIC QUALITY INSPECTED 8

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

2

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1. AGENCY USE ONLY (Leave blank)

2. REPORT DATE

October 1997

3. REPORT TYPE AND DATES COVERED

Annual (1 Oct 96 - 30 Sep 97)

4. TITLE AND SUBTITLE

Active Follow-Up of Participants of a Population-Based Specimen Bank (Plasma, DNA, RBC)

5. FUNDING NUMBERS

DAMD17-94-J-4265

6. AUTHOR(S)

Helzlsouer, Kathy J., M.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Johns Hopkins University
Baltimore, Maryland 212058. PERFORMING ORGANIZATION
REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-501210. SPONSORING / MONITORING
AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for public release; distribution unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT (Maximum 200 words)

From May through November 1989, 32,320 specimens were collected in Washington County, Maryland for a research specimen bank (CLUE II). At the time of blood collection participants gave a brief medical history, completed a food frequency questionnaire and returned a toenail clipping. The purpose of the follow-up study is to expand the resources of the existing population-based specimen bank by updating baseline information and obtaining information on breast cancer risk factors so that gene-environment interactions leading to breast cancer may be investigated.

CLUE II participants living within a 30-mile radius of downtown Hagerstown were included in the active follow-up cohort. This geographic boundary includes all of Washington County and parts of surrounding counties, extending into Pennsylvania and West Virginia. This boundary includes 30,724 of the 32,320 CLUE II participants. Persons under the age of 13 at the time of participation and persons known to have died, were excluded from the follow-up cohort leaving 28,410 in the study group. Of these, questionnaires were sent to 24,716 for whom addresses could be found. Questionnaires were returned by 16,301 participants for a response rate of 70%.

14. SUBJECT TERMS

Breast Cancer

15. NUMBER OF PAGES

41

16. PRICE CODE

17. SECURITY CLASSIFICATION
OF REPORT

Unclassified

18. SECURITY CLASSIFICATION OF THIS
PAGE

Unclassified

19. SECURITY CLASSIFICATION
OF ABSTRACT

Unclassified

20. LIMITATION OF ABSTRACT

Unlimited

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Kathy J. Johnson
PI - Signature

10/20/97
Date

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INTRODUCTION

Objective:

The purpose of this proposal was to expand the resources of our existing population-based specimen bank by updating baseline information and obtaining information on breast cancer risk factors so that gene-environment interactions leading to breast cancer may be investigated.

Background of Previous Work:

The CLUE Specimen Banks

From August through November, 1974, a total of 25,620 serum specimens were collected in Washington County, MD for a research specimen bank (CLUE I). An additional 182 specimens were obtained in the summer of 1975, for a total of 25,802. Linkage of the records from this program to those of a private census in the summer of 1975 indicated that almost a third of the adult population of the county had participated. Participation was best in the age group 35 to 65 years, and was slightly better among females, the better educated, and non-smokers.

A second program was conducted from May through November, 1989 collecting 32,898 blood specimens (CLUE II). Of these specimens, 9026 were collected from individuals who had also participated in CLUE I. CLUE II participants donated 20 ml of blood, gave a brief medical history, completed a food frequency questionnaire and returned a toenail clipping for trace metal studies. The blood specimens have been stored at -70 C. Somewhat greater participation was obtained among older persons, possible because a free cholesterol test was offered as an incentive to participate.

In addition to storing two 5 ml aliquots of plasma, another 0.7 ml aliquot was preserved with 0.7 ml of 10% metaphosphoric acid to allow subsequent ascorbic acid assays. The buffy coat (providing DNA) and an aliquot of red blood cells were also stored. The two aliquots of plasma from each person are stored in separate freezers.

Clue I has been extensively used to examine the potential protective effect of specific micronutrients against the development of cancer including breast cancer (1). The role of endogenous hormones in the development of breast cancer has also been examined using the resources of this serum bank (2). With the maturation of the cohort from Clue II, the availability of DNA in addition to plasma, and the technological advances of molecular biology we will be able to investigate etiologic, protective and

susceptibility factors leading to the development of breast cancer. In order to use the specimen bank to its fullest potential we require additional data on participants in the CLUE II cohort such as extended family history and other risk factors. Breast cancer risk factors such as family history of disease, age at pregnancy and parity change over time requiring institution of active follow-up of the cohort. These risk factors should be taken into account in investigations of serologic precursors of susceptibility factors associated with breast cancer. The ability to identify and investigate families with multiple members affected by breast cancer is a valuable resource for studying the role and contribution of inherited susceptibility factors to the development of breast cancer. Since these inherited factors may be passed through the mother or father it is essential to obtain information on the family history of cancer of male and female participants in the cohort.

Stability studies of micronutrients and hormones have been completed up to 42 months. The resources of serum and specimen banks become more valuable with time as the number of cancer cases developing among participants increases with aging of the cohort. It is essential to assess the stability over many years of factors being studied in order to optimally utilize these valuable resources. A literature review of the effects of long-term freezer storage on concentrations of retinol, beta-carotene, and alpha-tocopherol in repeated assays of the same serum or plasma pools shows that most of the studies are deficient because of very small numbers of observations, imprecise descriptions of procedures, and/or short periods of storage (3). The literature is even more scanty with respect to long term storage effects on concentrations of other carotenoids, ascorbic acid, and hormones. It is essential to have this information to satisfy study sections and reviewers of manuscripts. In our experience over the past 20 years, such queries have been almost universal.

Cancer Register:

A cancer register for Washington County has been maintained since 1958, with records dating back to 1948. Its primary source is discharge records from the Washington County Hospital, the only general hospital in the county. Because of its well-equipped and staffed Oncology Service, the hospital tends to draw patients from surrounding counties rather than to lose them to other institutions. Cases are also ascertained from death certificates of Washington County residents which are under the custody of our unit acting as a branch of the Health Department. Comparisons of observed cases in the populations that donated blood for the serum bank with the number expected on the basis of race-sex age specific rates from the SEER registries suggest that reporting is essentially complete for this sub-population. The only major deficit is for stomach cancer; the only major excess is for

prostate cancer. Records of reported county cancer cases are computer-linked to the lists of serum bank donors. Matching on variables such as age, date of blood donation, day of menstrual cycle are readily accomplished. Age matching is often possible within a few days or weeks.

Purpose of Present Work

Further progress in understanding the etiology of breast cancer and in developing new methods for early detection and prevention requires investigators to consider other genetic and environmental influences and their interaction in the development of breast cancer. The purpose of our project is to expand the resources of our existing population-based specimen bank by updating baseline information and obtaining information on breast cancer risk factors, particularly family history of cancer. Gene-environment interactions may be explored to the fullest potential. The availability of family history information will permit the targeting of high risk individuals, based on familial factors, for investigations of possible inherited susceptibility factors.

The second major objective of this proposal is to continue to obtain fundamental information on changes in the concentration of various analytes in plasma and blood cells associated with long-term storage at -70 C. The plasma analytes are retinol, ascorbic acid, the major carotenoids found in human serum, alpha- and gamma-tocopherol, sex-hormone-binding globin, and selected steroid hormones. The hypothesis to be tested in each case is the null hypothesis that there will be no change in concentration with storage time. The resources of banks become more valuable with time as cohorts mature providing more cases of cancer for investigation, and as new hypothesis and techniques become available for evaluation. Storage studies will provide basic information on the stability of markers of exposure, susceptibility and protection from breast cancer that is almost nonexistent. This information is essential for planning and interpreting studies using stored plasma.

Technical Objectives (Specific aims)

To enhance the resources of the existing population-based specimen bank we propose to:

1. Expand information collected at CLUE II in 1989. For example, total years of smoking and medication use, especially exogenous hormones.
2. Update information collected at CLUE II. For example, changes in marital status, smoking status, use of exogenous hormones, weight changes and dietary changes.

3. Obtain additional information relevant to breast cancer. For example, detailed cancer family history, number and timing of pregnancies, preventive health behavior (breast cancer screening), history of breast biopsies and occupational exposures.
4. Expand the cancer registry population base by including CLUE II participant who reside outside of Washington County.

To facilitate the appropriate use of the resources and to enhance interpretation of studies performed on the cohort we plan to:

5. Continue the study of the effects of long-term freezer storage of plasma at 70 C on its content of antioxidant nutrients, such as retinol (including retinoic acid and retinol palmitate), ascorbic acid, carotenoids, and tocopherols, as well as its content of hormones and lipoproteins.

Methods

Eligible Population

Clue II participants living within a 30 mile radius of downtown Hagerstown were included in the active follow-up cohort. This geographic boundary includes all of Washington County and parts of surrounding counties, extending into Pennsylvania and West Virginia. This boundary includes 30,724 of the 32,898 CLUE II participants. This boundary was chosen to form a homogenous cohort for long-term follow-up. Of the CLUE II samples excluded from the cohort, 2,200 will be used for our storage studies and cross-sectional investigations that can be conducted using data collected at baseline. Persons under the age of 13 at the time of participation and persons known to have died, were excluded from the follow-up cohort.

Follow-up Procedure

CLUE II participants located in the geographic boundary of the long-term follow-up cohort will be mailed a questionnaire approximately every 18-24 months. It will take a considerable amount of clerical work to update addresses from the time of CLUE II participation since all rural routes box numbers have been changed to street (road) addresses.

Non-respondents will be sent a post card reminder followed by a second questionnaire. Telephone follow-up will be carried out to clarify questionnaires completed by respondents and to administer the questionnaire to non-respondents as resources permit. The resources available to search for current addresses of participants

include the Polk's City Directory for Hagerstown which covers Washington County and surrounding communities, telephone directories, and the Hill-Donnelly Cross Reference Directory.

As part of this study, we proposed to collect additional data on CLUE II participants. Specifically, data will be collected from women on known risk factors for breast cancer. Examples of risk factors include: family history of cancer in first and second degree relatives, history of breast biopsy, type of benign breast disease, age at menarche, first birth, menopause, parity, months of lactation, use of exogenous hormones, height and weight and screening history. Updated risk factor information will be obtained at approximately 18-24 month intervals. Updated food frequency information will be obtained at the second mailing. Because inherited susceptibility factors may be inherited through the mother or the father and because of recent evidence of common inherited and environmental factors for breast and prostate cancer we will include male and female participants in the active follow-up cohort.

Information will be obtained from participants using a self-administered mailed questionnaire. A post card followed by a second questionnaire will be sent to non-respondents. Deceased CLUE II participants will be identified by linking the population to the mortality data base and by using obituaries from the local newspaper. The Training Center for Public Health Research is a repository for county death certificates.

Machine readable forms will be used for all questions except family history. Questions related to family history are not practical in a machine readable format because name, age at diagnosis, and all causes of death must be recorded. This information will be hand entered.

As part of the questionnaire development controlled trials of the effect of length, incentives, and follow-up techniques on response to a mailed questionnaire were conducted. Interventions tested included variations on length of the questionnaire, effect of a non-monetary incentive and effect of a postcard reminder versus a letter accompanied by a second questionnaire. Response rates were similar for short (16 questions, 4 pages) and long (77 questions, 16 pages) questionnaire groups. The non-monetary incentive did not improve response rates. The second mailing of a questionnaire was significantly better than a postcard reminder in improving response (23 percent versus 10 percent). A paper has been submitted for publication of these findings.

Methods for Studies of Stability

The campaign for participation in CLUE II was conducted from May to November 1989. Blood was drawn into a 20 ml vacutainer containing heparin and was refrigerated at 4 C until it was delivered to the CLUE II laboratory within a few hours after being drawn. After centrifugation, 0.7 ml of plasma was divided into two equal aliquots, the buffy coat was removed, as were 2 ml of red blood cells. Each of these specimens were placed in cryotubes and promptly frozen at -70 C.

For the stability studies of micronutrients and hormones, pools of plasma were created from the plasma of persons who lived outside of the study area and who had donated blood near the end of the campaign. For the micronutrients, 40 pools were created, each containing the plasma from four individuals. The 40 micronutrient pools and the 16 hormone pools were each composed of equal numbers of pools from young and old men, and young and old women. A large reference pool was also created so that four aliquots could be added to the micronutrient specimens and to the hormone specimens.

Assays for micronutrients and hormones have been done at baseline, 12, 22, and 42 months after the median time of blood drawing. We propose to do three more rounds of assays. The assays will be done in the same laboratories at each round, using the same methods insofar as this is practical. Micronutrient assays have been done in the laboratory of Dr. Edward Norkus at Our Lady of Mercy Medical Center in New York City using high performance liquid chromatography. The micronutrients assayed included retinol, alpha-and gamma-tocopherol, the carotenoids, and ascorbic acid. The carotenoids included total carotenoids, alpha-and beta-carotenes, cryptoxanthin, lutein, and lycopene (4). Ascorbic acid was assayed by Dr. Norkus on plasma preserved with meta-phosphoric acid using high performance liquid chromatography (5). The hormone assays have been done in the laboratory of Dr. Christopher Longcope, University of Massachusetts. Assays include estrone, estradiol, testosterone, and progesterone (6). Inter-and intra-assay coefficients of variation for these assays are approximately 10% and 7% respectively. Sex hormone binding globulin capacity will be measured using the filter disc method of Mickelson and Petra (7). Inter-and intra-assay coefficients of variation are reported to be 10.9% and 8.0% respectively (8).

The basic analysis of the data was to calculate regression lines for each pool and to average the slopes of each age-sex group, and when appropriate, for the total group. This approach assumes that an equal amount of analyte is lost in each unit of time. Other assumptions above the rate of change can be made, but have not been deemed necessary.

BODY: PROGRESS REPORT YEAR 3**Tasks 3,5: Data Entry and Scanning of Questionnaire; Editing and Telephone Clarification of Responses**

Data entry and editing for the first round questionnaire mailing is complete. Preliminary tabulations have been done for all questions. Family history data has been entered and tabulated (Table 2). Data has been linked to the cancer registry (Table 4). A study of persons reporting cancer who are not in the registry is underway. Appendix 1 shows the overall tabulations from the first round questionnaire for some of the known risk factors for breast cancer. Appendix 2 shows self-reported breast cancer cases by age at diagnosis. Appendix 3 shows the conditions reported by respondents to the first round CLUE II follow-up questionnaire by sex and age at diagnosis.

Task 3: Follow-up of Non-Respondents

During the first week of October 1996 a second questionnaire was mailed to approximately 11,000 non-respondents. After 21 days 20 percent had responded.

In July of 1997, after data entry and scanning of questionnaires was completed, we began a telephone follow-up of non-respondents. The follow-up group was limited to Washington County residents at baseline (CLUE II, 1989) who currently resided in the county, and for whom we had a telephone number listed in our data base. We established this inclusion criteria so that we could contact as many persons as possible in a limited time period, and reduce costs by eliminating the need for long distance calls. Lists of non-respondents were printed which included the name, address, date of birth and telephone number of the participant. Lists were distributed to staff according to alpha-numeric participant number. Staff were instructed to make one attempt to contact the participant. Contact was made with 2,135 persons. Of that group a shortened version of the questionnaire was administered over the telephone to 759 persons. If the participant refused the phone interview we asked if we could send a questionnaire which could be completed at his or her leisure. Eight-hundred and eighty-two questionnaires were mailed of which 238 questionnaires were returned. The response rate for the mailed questionnaires was 27 percent. Two-hundred and forty-five persons refused. We were unable to obtain a current address for 182 persons. Overall response for the follow-up group was 53 percent. See Appendix 4 for a summary of the follow-up population.

Task 5: Preliminary Tabulations of Responses

Questionnaires were returned by 16,301 participants for a response rate of 70 percent. Age and education were associated with response rate, with the highest response being among persons the 65-74 age group (81 percent), and among those with 13 or more years of education (75 percent), compared to 62 percent among persons with less than 12 years of education. Response was higher among females (73 percent) versus 66 percent for males, and among married persons (73 percent) compared to 55 percent for persons who were never married. Overall, response was higher among persons residing within Washington County (73 percent) compared to those living outside of the county (57 percent). Tables 5-7 give an overview of response, non-response and CLUE II participants that we were unable to locate by age, sex, education and geographic location.

Task 9: Study of effects of stability of plasma components when stored at -70 C

Participants in CLUE II donated 20 ml of blood in 1989. This was collected in a 20 ml vacutainer containing heparin and was stored at 40 C until it was delivered to the laboratory within a few hours after being drawn. After centrifugation, 0.7 ml of plasma was added to 0.7 ml of 10% metaphosphoric acid for ascorbic acid assays. The remaining plasma was divided into two equal aliquots, the buffy coat was removed, and 2 ml of red blood cells was saved. Each of these specimens was placed in 5 ml cryotubes and promptly frozen at -70 C.

For the stability studies of micronutrients, and hormones, pools of plasma were created from specimens donated by persons who lived far outside the study area and who had participated near the end of the campaign. For ascorbic acid assays, 40 pools were created, each containing the plasma from four individuals. For the other assays, 16 pools were created, again each containing the plasma from four persons. For all of the analytes, one quarter of the pools was composed of plasma from each of the four sex-age groups (males born 1910-1939, males born 1949-1969; females born 1910-1939; and females born 1940-1969). A large quality control pool was also created so that four quality control aliquots could be included in the ascorbic acid assays, and two in the other assays.

Ascorbic acid assays were done after 12, 24, and 42 months of freezer storage. Other micronutrients were assayed after 15.5, 27.5 and 51.5 months. These results have been reported (9). Briefly, they show that there was no indication of any meaningful losses of ascorbic acid, retinol, alpha-tocopherol, or gamma-tocopherol during the approximately 4 years of storage. A fourth series of assays has just been completed.

The situation with respect to hormones is unclear, to put it mildly. Estrone, estradiol, progesterone, testosterone, and sex hormone binding globulin were assayed after 6, 12, 31, and 78 months of storage. The mean values for all 16 aliquots are shown in table 1 along with minimum and maximum values at each time and the coefficients of variation for the two quality control specimens. Most of these coefficients are within reasonable limits, although six of the 20 exceed 15 %. Most troubling are the marked increases in concentration with long-term storage for estrone and estradiol, and the sharp drop at 78 months in the concentration of progesterone. There has been no evidence of loss of water during storage. We plan to re-assay aliquots of serum or plasma previously assayed by the same laboratory that has done the storage effects study. If the finding of marked changes is confirmed, this will have major consequences for studies of hormones in stored plasma or serum.

CONCLUSIONS

Plans for the next year are as follows:

- Task 4:** 1. Preparation and mailing of the annual newsletter for the second round questionnaire.
- Task 6:** 2. Updating of address information and verification of vital status in preparation for mailing of the second questionnaire.
- Task 6:** 3. Development and printing of second follow-up questionnaire. Pilot testing of second follow-up questionnaire.
- Task 6:** 4. Mailing of second follow-up questionnaire.
- Task 6:** 5. Follow-up of non-respondents via a post card reminder with subsequent mailing of a second questionnaire.
- Task 6:** 6. Telephone follow-up of non-respondents.
- Task 6:** 7. Data editing and optical scanning of returned questionnaires.
- 8. Preliminary tabulations of data from second round questionnaire.
- Task 10:** 9. In the fall of 1998, nine years after blood was drawn, aliquots of the 40 pools for micronutrient assays will be examined for the same micronutrients and hormones as in the first four rounds.

- * See Appendix 5 which gives a detailed study time schedule. Note that the time schedule exceeds the funding period due to a delay in start-up of the project. We anticipate that we will complete the work with the level of funding that was previously awarded.

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Table 1

Mean values, standard deviations, minimum and maximum values
for selected hormones in 16 different plasma pools assayed
after 6, 12, 31, and 78 months of storage at -70° C,
Clue II, Washington County, MD

<u>Months of storage</u>	<u>Summary values for 16 pools</u>				<u>Coeff. of variation*</u>
	<u>Mean</u>	<u>S.D.</u>	<u>Minimum</u>	<u>Maximum</u>	
<u>ESTRONE</u> (pg/ml)					
6	33.0	13.0	16	54	8.1
12	24.1	9.0	12	42	5.7
31	45.0	21.2	16	93	19.4
78	73.5	24.3	41	121	29.4
<u>ESTRADIOL</u> (pg/ml)					
6	28.0	15.2	11	64	10.9
12	19.9	9.6	7	44	12.5
31	56.4	28.6	30	128	3.9
78	55.4	32.1	28	134	52.9
<u>PROGESTERONE</u> (ng/ml)**					
6	4.06	3.43	1.60	8.92	8.7
12	4.80	2.61	1.05	6.74	63.6
31	4.24	3.74	1.06	9.26	6.2
78	1.24	0.51	0.68	1.84	29.4
<u>TESTOSTERONE</u> (ng/ml)					
6	2.25	2.41	0.03	6.66	4.1
12	1.98	1.92	0.16	5.72	10.4
31	2.91	2.82	0.18	7.29	2.3
78	2.48	2.51	0.16	6.71	4.2
<u>SEX HORMONE BINDING GLOBULIN</u> (nmol/L)					
6	56.4	22.9	28.5	99.8	7.9
12	55.7	21.7	28.9	95.5	16.5
31	54.5	19.2	29.4	96.0	4.1
78	49.3	17.0	29.7	87.3	1.0

* In %, based on 2 aliquots of quality control plasma from same pool.

** Based on the four pools from young females (ages 20-49 years). Other age-sex groups were excluded because values were extremely low and often below the level of detection.

Table 2

**Family history of breast cancer reported by respondents to Clue II follow-up
questionnaire 1996-1997**

		Number	% of respondents
Women	Mother	514	5.6
	Sister	469	5.1
	Mother or sister	944	11.4
	Mother and sister	39	0.5
Men			
	Mother	247	4.6
	Sister	275	4.6
	Mother or sister	514	9.6
	Mother and sister	8	0.1

Table 3

**Change in body mass index from Clue II (1989) to the active follow-up
(1996-1997)**

	<u>mean</u>	<u>median</u>
baseline	25.8	24.9
Women 1996	27.0	25.9
difference	1.2	0.9
baseline	27.9	26.2
Men 1996	28.5	26.7
difference	0.6	0.7

Table 4

**Linkage of self-reported cancer cases with the cancer registry
Washington County residents**

Site	Num Self Reported	% on Registry
Breast	282	85
Cervix	144	51
Uterus	140	70
Ovary	53	74
Colon	126	90
Lung	43	84
Melanoma	213	40
Basal Cell	709	34
Squamous	197	42
Prostate	188	87

Table 5

ACTIVE FOLLOW-UP OF CLUE II PARTICIPANTS

Number (and percent) **response** by age, sex,
education and geographic location of
total sample in **first** questionnaire mailing

	<u>No. in sample</u>	<u>Response rate, %</u>
Total	16301	69.8
<u>Age (years)</u>		
<25	413	48.4
25-34	1106	52.7
35-44	2379	61.7
45-54	3414	67.7
55-64	3404	76.9
65-74	3481	81.3
75+	2104	74.9
<u>Sex</u>		
Male	6395	65.5
Female	9906	72.8
<u>Education (years)</u>		
<8	1191	62.0
9-11	1945	61.3
12	7374	69.7
13+	5774	75.3
Missing	17	--
<u>Geographic location</u>		
Washington County	13879	72.5
Outside of Washington County	2422	57.2

Table 6

ACTIVE FOLLOW-UP OF CLUE II PARTICIPANTS

Number (and percent) **non-response** by age, sex,
education and geographic location of
total sample in **first** questionnaire mailing

	<u>No. in sample</u>	<u>Non-response rate, %</u>
Total	7069	30.2
<u>Age (years)</u>		
<25	441	51.6
25-34	991	47.3
35-44	1480	38.4
45-54	1631	32.3
55-64	1020	23.1
65-74	802	18.7
75+	704	25.1
<u>Sex</u>		
Male	3368	34.5
Female	3701	27.2
<u>Education (years)</u>		
<8	731	38.0
9-11	1229	38.7
12	3205	30.3
13+	1898	24.7
Missing	3	--
<u>Geographic location</u>		
Washington County	5257	27.5
Outside of Washington County	1812	42.8

Table 7

ACTIVE FOLLOW-UP OF CLUE II PARTICIPANTS

Number (and percent) **unable** to locate by age, sex,
education and geographic location of total
sample in **first** questionnaire mailing.

	No. in <u>sample</u>	Unable to locate <u>Rate %</u>
Total	4796	17.0
<u>Age</u> (years)		
<25	3809	81.7
25-34	257	10.9
35-44	284	6.9
45-54	207	3.9
55-64	116	2.6
65-74	75	1.7
75+	48	1.7
<u>Sex</u>		
Male	1998	17.0
Female	2798	17.1
<u>Education</u> (years)		
<8	360	15.8
9-11	752	19.2
12	1966	15.7
13+	1712	18.2
Missing	6	--
<u>Geographic location</u>		
Washington County	3560	15.7
Outside of Washington County	1236	22.6

Table 8

Changes in smoking behavior from Clue II (1989) to the active follow-up

Ever smoke cigarettes?

	<u>Baseline %</u>	<u>Follow-up %</u>
Women	35.2	40.2
Men	54.6	58.3
All	42.9	47.4

Current smokers in 1989 who reported stopping for at least 6 months in the active follow-up

% of 1989 smokers

Women	59.1
Men	40.9
All	56.7

Number of cigarettes per day reported by those who reported ever smoking

	<u>Baseline %</u>	<u>Follow-up %</u>
< 1	1.9	7.7
1-4	9.0	14.8
5-14	21.6	25.6
15-24	40.0	31.6
25-34	11.1	11.7
35 +	17.5	8.6

Table 9

Changes in marital status from Clue II (1989) to the active follow-up

<u>Baseline Status</u>	Status reported in the active follow-up				
	<u>Nev</u>	<u>Mar</u>	<u>Wid</u>	<u>Div</u>	<u>Sep</u>
never married	66.3	29.7	0.2	2.7	1.1
married	0.1	90.6	5.3	2.6	1.3
Other	1.6	13.7	48.0	34.8	1.9

Appendix 1

**Prevalence of breast cancer risk factors reported by female respondents to
Clue II follow-up questionnaire 1996-1997**

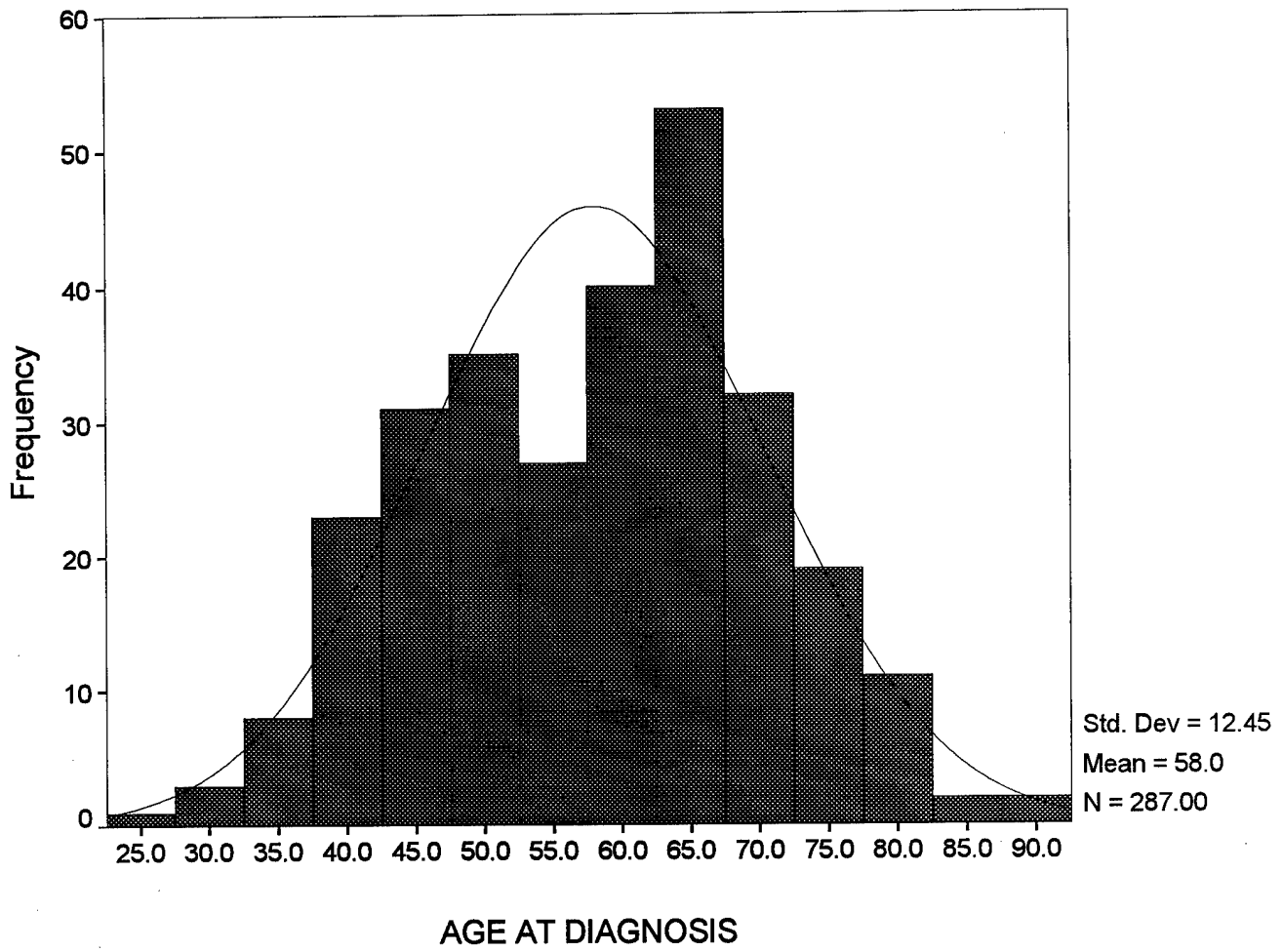
<u>Risk Factor</u>	<u>Number</u>	<u>% of respondents</u>
Estrogen use	2667	29.8
Years Used		
<1	183	2.1
1-5	1200	13.7
6-10	519	5.9
11-15	277	3.2
16-20	146	1.7
21-25	99	1.1
26 +	111	1.3
(mean = 7.6)		
Progestin use	1122	12.8
Years Used		
<1	133	1.5
1-5	618	7.1
6-10	203	2.3
11-15	55	0.6
16 +	33	0.4
(mean = 4.4)		
Birth Control Pills	4316	47.9
Years Used		
<1	134	1.5
1-5	2167	24.6
6-10	1179	13.4
11-15	438	5.0
16 +	224	2.5
(mean = 6.2)		
Parity		
Num of Children		
0	1366	15.4
1-3	6167	69.6
2-6	1217	13.7
>=7	112	1.3
(mean=2.5)		

Appendix 1 (continued)

<u>Risk Factor</u>	<u>Number</u>	<u>% of respondents</u>
Age at first birth		
Age Group		
<20	1977	22.3
20-29	4960	56.0
30+	587	6.6
mean=22.6		
Breast fed children	3367	37.3
Months Breastfeeding		
1-12	2150	24.6
13-24	591	6.7
25-36	239	2.7
over 36	176	2.0
Menstrual History		
Age first menses		
<=11	1692	18.7
12-14	6290	69.4
>=15	1077	11.9
mean age = 12.7		
Menopause Age		
<45	2524	30.0
45-54	2780	33.0
>=55	511	6.1
Family History of Breast Cancer		
Mother	514	6.2
Sister	469	5.2
Mother or sister	944	11.4
mother and sister	39	0.5
BMI > 27	3691	41.7
BMI < 21	999	11.3
Oophorectomy before age 40	789	9.8
Alcohol 2 + drinks/day	315	3.6
History of Cancer Uterus (without breast cancer)	145	1.6
History of Cancer ovary (without breast cancer)	51	0.6

REPORTED BREAST CANCER CASES

CLUE II ACTIVE FOLLOW-UP



Conditions reported by respondents to CLUE II follow-up questionnaires,
by sex and by age at diagnosis, 1996-1997*

Condition	Total	No.	% Female	% Male	Age at diagnosis (%)					
					Female			Male		
					<40	40-59	60+	<40	40-59	60+
a. Diabetes	7.5	1122	6.9	8.3	2.0	5.5	10.3	1.6	6.2	12.2
b. High cholesterol	35.7	5383	35.6	35.9	12.3	31.0	49.1	15.3	36.6	41.4
c. Heart attack	5.9	890	3.3	9.9	0.1	1.3	6.5	0.2	4.7	17.4
d. Angina pectoris	5.0	750	4.3	6.1	0.1	1.7	8.3	0.1	2.6	11.2
e. Stroke	2.1	314	1.8	2.5	0.1	0.7	3.5	0.0	0.7	4.9
f. TIA	1.6	239	1.6	1.7	0.1	0.4	3.2	0.0	0.5	3.2
g. Peripheral artery disease	2.5	367	2.0	3.1	0.0	1.2	3.6	0.1	1.2	5.8
h. Osteoporosis	5.1	764	7.9	0.9	0.3	3.1	15.3	0.4	0.7	1.3
i. Hip fractures	1.3	192	1.5	0.9	0.5	0.6	2.9	0.5	0.7	1.3
j. Wrist fracture	5.6	840	5.8	5.4	3.3	3.3	9.1	5.1	5.1	5.7
k. Fibrocystic disease	16.9	2521	27.7	--	13.2	38.4	23.4	--	--	--
l. Endometriosis	5.6	831	9.2	--	7.9	12.9	6.2	--	--	--
m. Uterine fibroids	7.9	1176	13.0	--	4.3	17.5	12.2	--	--	--
n. High blood pressure	30.0	4512	28.8	31.9	6.6	22.2	43.9	9.7	27.2	42.9
o. Migraine headaches	13.4	2017	17.8	6.8	18.3	21.7	13.9	8.9	8.3	4.8
p. Thyroid disease	7.4	1112	10.9	2.2	3.9	9.4	15.0	0.4	1.5	3.3
q. Rheumatoid arthritis	7.6	1141	8.5	6.2	1.2	6.5	13.4	1.2	4.4	9.4
r. Gallbladder disease	11.2	1677	14.3	6.4	4.3	13.0	19.5	0.9	4.2	10.0
s. Gastric or duodenal ulcer	7.9	1179	7.4	8.6	3.5	7.0	9.4	3.2	6.3	12.2
t. Macular degeneration	1.9	288	2.1	1.7	0.0	0.6	4.2	0.1	0.7	3.2
u. Cataract	12.6	1891	13.5	11.4	0.3	1.9	29.7	0.5	2.4	22.1
v. Asthma	7.9	1186	8.8	6.5	10.3	9.5	7.6	9.3	7.2	5.1
w. Emphysema/Chron bronchitis	6.2	937	6.6	5.8	2.9	6.2	8.3	1.6	3.7	8.9
x. Diverticulitis	6.1	911	6.9	4.9	0.8	3.7	12.2	0.1	3.3	7.8

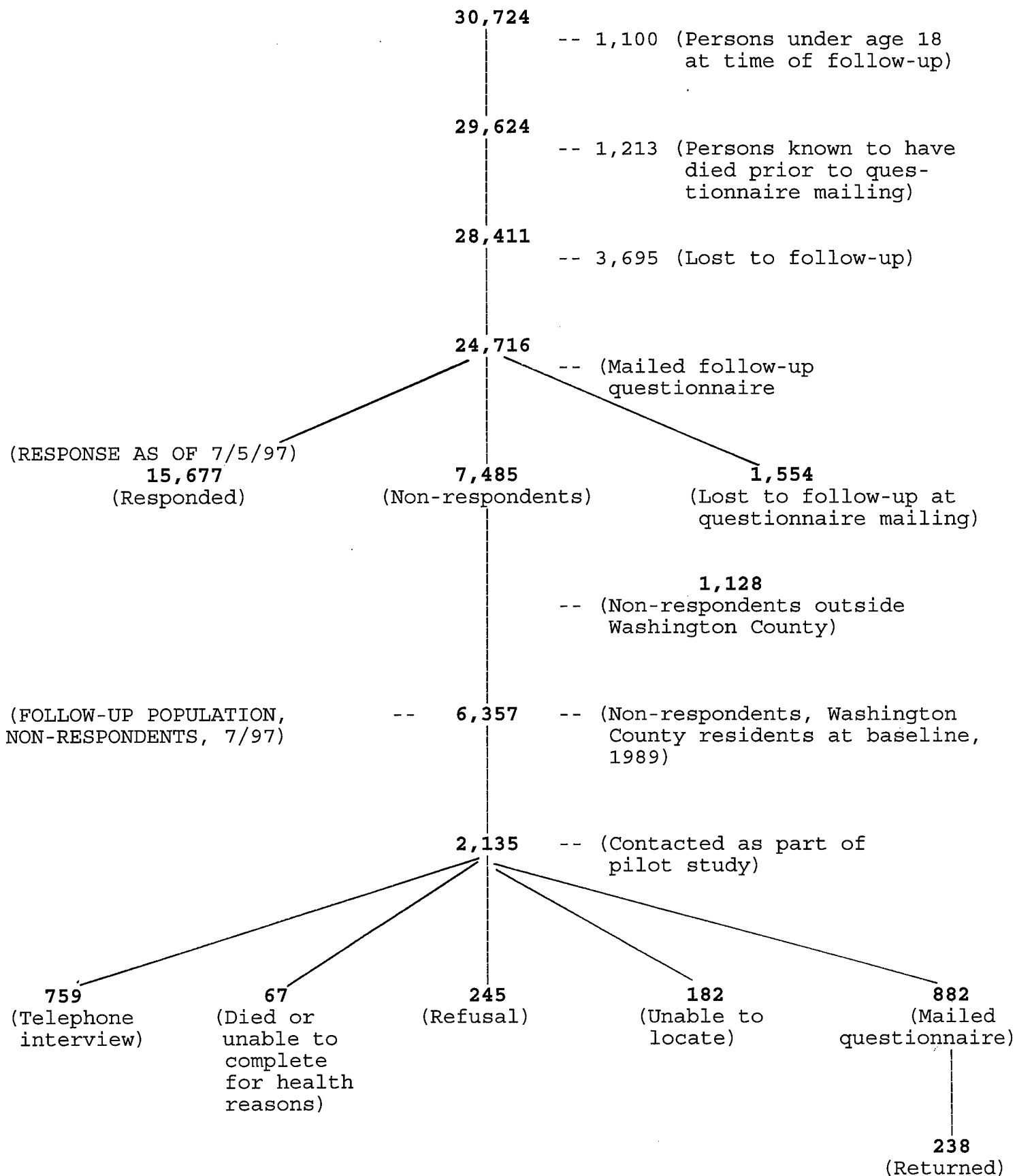
Appendix 3 (continued)

- 2 -

Condition	Total	No.	% Female	% Male	Age at diagnosis (%)					
					Female			Male		
					<40	40-59	60+	<40	40-59	60+
y. Parkinson's disease	0.4	57	0.3	0.5	0.0	0.1	0.6	0.0	0.2	0.9
z. Kidney stones	8.3	1240	5.3	12.8	3.1	5.4	5.9	3.8	12.8	15.6
aa. Ulcerative colitis	1.3	195	1.4	1.5	0.9	1.5	1.6	0.7	0.9	2.2
bb. Cancer of breast	2.1	311	3.4	0.1	0.1	2.0	6.0	0.0	0.0	0.2
cc. Cancer of cervix	--	163	1.8	--	1.9	1.9	1.7	--	--	--
dd. Cancer of uterus	--	160	1.8	--	0.3	1.4	2.7	--	--	--
ee. Cancer of ovary	--	59	0.6	--	0.2	0.4	1.1	--	--	--
ff. Colon or rectal polyp	7.1	1059	6.3	8.2	0.9	4.4	10.3	0.6	4.9	13.6
gg. Cancer of colon or rectum	1.0	149	0.8	1.3	0.0	0.3	1.6	0.0	0.3	2.6
hh. Cancer of lung	0.3	48	0.2	0.4	0.0	0.1	0.5	0.1	0.3	0.7
ii. Melanoma	1.7	255	1.4	2.1	0.2	1.2	2.2	0.1	1.2	3.5
jj. Basal cell skin cancer	5.5	828	4.8	6.6	0.5	3.1	8.2	0.2	3.2	11.7
kk. Squamous cell skin cancer	1.5	222	1.0	2.2	0.1	0.6	1.7	0.1	0.6	4.4
ll. Cancer of prostate	--	211	--	3.5	--	--	--	0.0	0.5	7.2
mm. Other cancer (specify)	2.4	360	2.1	2.9	0.9	1.6	3.2	0.9	1.8	4.7

* Study population includes persons living within 30 miles of intersection of I-70 and I-81.

**ACTIVE FOLLOW-UP OF PARTICIPANTS OF
A POPULATION-BASED SPECIMEN BANK**
Summary of Follow-up Population



Appendix 5

10/2/97

TIME SCHEDULE

October 1997	Complete draft questionnaire development. Identify vendor to print form. Complete mock-up of questionnaire. Print questionnaire for Pilot Study. Order supplies for Pilot. Continue preparation of newsletter. Identify vendor to print and mail newsletter.
November 1997	Pilot testing of questionnaire Set up data entry tables for Pilot. Data entry of Pilot questionnaire. Modifications of questionnaire based on Pilot testing. Continue newsletter development.
December 1997	Complete data entry of Pilot. Modify questionnaire based on Pilot. Review mock-up of newsletter. Set up computer tables for second round questionnaire mailing.
January 1998	Mail newsletter Complete address corrections from newsletter mailing.
February 1, 1998	Final printing of second round questionnaire.
March 1998	Begin mailing of second round questionnaire.
April 15, 1998	Complete mailing of second round questionnaire.
April 22, 1998	Postcard reminders to non-respondents.
May 15, 1998	Mail second questionnaire to non-respondents.
May-August 1998	Editing and scanning of second round questionnaire.
September 1998	Complete data entry and scanning. Data editing and management. Preliminary tabulations.

Appendix 5 (continued)

Sept. 1998-Jan 1999	Development of third round questionnaire.
January 1999	Pilot testing of third round questionnaire.
March 1999	Modifications and final printing of third round questionnaire. Set up computer tables and form descriptions for third round questionnaire.
April-August 1999	Begin to order supplies for mailing. Order reminder postcards. Prepare address tables. Print labels.
Sept.-Oct. 1999	Mailing of third round questionnaire. Two weeks after mailing send post card reminder.
Oct.1999-March 2000	Complete data entry and scanning. Data editing and data base management.

DRAFT SECOND ROUND QUESTIONNAIRE





Study Number _____

INSTRUCTIONS

THIS FORM IS DESIGNED TO BE USED BY OPTICAL SCANNING EQUIPMENT.

1. Please use an ordinary No. 2 pencil to answer all questions.

(Picture of pencil here)

2. Make heavy black marks that darken the circle completely. Please do not mark this way:
- 


- Please mark this way: 
3. If you change your mind, please erase completely.
4. Unless the instructions tell you otherwise, darken only one circle.
5. Note that some questions ask for information by certain time periods and some ask for current status.

EXAMPLE: Mark the "Yes" circle and Year of Diagnosis circle for each illness you have had diagnosed.

Have you been told by a doctor or other health professional that you have any of the conditions listed below?

		<u>Year of first diagnosis</u>			
		1989		After	
		Before		to	
		1989		July 1, 1996	
Leave blank for "No". Mark here for "Yes":					
Diabetes mellitus	<input checked="" type="radio"/> -->	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Elevated cholesterol	<input type="radio"/> -->	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
High blood pressure	<input checked="" type="radio"/> -->	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input checked="" type="radio"/>

6. If you have comments, please write them on the last page of the booklet.

PLEASE CHECK THE INFORMATION BELOW AND CORRECT THE INFORMATION IF THERE IS A MISTAKE.

If the person whose name appears on this form is deceased, please STOP HERE and provide the date of death: _____. Please return the blank questionnaire in the postage-paid envelope.

THANK YOU FOR COMPLETING THE QUESTIONNAIRE. PLEASE RETURN THE BOOKLET IN THE ENCLOSED POSTAGE-PAID ENVELOPE.

HEALTH HISTORY

1. What is your date of birth?

Please write below

Month	Day	Year

Please Fill in the Circles

2. Have you been told that you have cancer?

☐ No

☐ Yes ---> Year of Diagnosis

☐ Before 1989

☐ Between 1989 and July 1, 1996

☐ After July 1, 1996

3. If you had cancer, what type of cancer did you have?
Please mark below.

TYPES OF CANCER										Please specify "other" type of cancer below	Age at diagnosis of first cancer (excluding skin cancer)									
Bladder	Breast	Cervix	Colon or rectum	Leukemia	Lung	Lymphoma or Hodgkins	Melanoma	Ovary	Pancreas		Prostate	Skin (basal or squamous)	Uterus or endometrium	Other or unknown	Under 20	20-39	40-49	50-59	60-69	70 or over
<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>

4. Where was the cancer diagnosed?

- ☐ Washington County Hospital
 - ☐ Other, specify place, city, state
-

5. Have you been told by a doctor or other health professional that you have any of the conditions listed below?

Leave blank for "No"
Mark here for "Yes"

(If you do not recognize the name of the condition, you probably haven't had it.)		Before 1989	1989 to July 1, 1996	After July 1, 1996	Where was the diagnosis made? Place, city, state
a. Diabetes	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	_____
b. Heart attack	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	_____
c. Angina pectoris	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	_____
d. Stroke	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	_____
e. Peripheral artery disease (pain with walking or exercise) (not varicose veins)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	_____
f. Osteoporosis	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	_____
g. Hip fracture	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	_____
h. Wrist fracture	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	_____
i. Fibrocystic disease of the breast or other benign breast disease (not cancer)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	_____
j. Endometriosis	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	_____
k. Uterine fibroids	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	_____
l. Macular degeneration of the retina	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	_____
m. Cataracts	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	_____
n. Colon or rectal polyps (benign) (not cancer)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	_____
o. Other major illness (specify illness)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	_____

7. During the past 10 years (since the time you donated to CLUE II), have you taken any of the following medications for your heart or blood pressure?

	No	Yes, but not currently	Yes currently
Calcium Blocker For example: Procardia, Cardizem, Norvase, Calan, Adalat, Sudar, (verapamil, amlodipine), etc.	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Beta Blocker For example: Lopressor, Tenormin, Inderol, (atenolol, metoprolol), etc.	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
ACE Inhibitor For example: Vasotec, Zestril, Capoten, Prinivil, Lotensin, Accupril, Monopril, (captopril) etc.	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Diuretic For example: Lasix, Lozol, (triamterene, HCTZ, furosemide, thiazides), etc.	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Other (mark here if unsure of name of heart or blood pressure medica- tion category) Specify medicine _____	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>

SMOKING

8. How many cigarettes do you usually smoke each day at the present time?

- | | |
|---|----------------------------------|
| <input type="radio"/> Do not smoke cigarettes | <input type="radio"/> 15-24 |
| <input type="radio"/> Less than 1 per day | <input type="radio"/> 25-34 |
| <input type="radio"/> 1-4 | <input type="radio"/> 35 or more |
| <input type="radio"/> 5-14 | |

9. Do you now use any of the following other tobacco products?

- | | |
|------------------------------|---------------------------------------|
| <input type="radio"/> Cigars | <input type="radio"/> Chewing tobacco |
| <input type="radio"/> Pipes | <input type="radio"/> None |
| <input type="radio"/> Snuff | |

10. Have you ever used "nicotine gum" or a "nicotine patch" to try to quit smoking?

- ☐ No
☐ Yes, nicotine gum only
☐ Yes, nicotine patch only
☐ Yes, both nicotine gum and nicotine patch

EXERCISE HISTORY

11. This question asks about exercise during high school and young adulthood. During these ages, on average about how many months during the year did you take part in moderate or strenuous (aerobic) physical activity or sports at least twice per week?

(Examples are swimming, aerobics, field hockey, basketball, cycling, and running; farm chores; brisk walking; other strenuous work)

a. During high school

b. During ages 18-22

	Months per year			
	Less than 4	4-6	7-9	10-12
	Never			
a. During high school	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
b. During ages 18-22	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>

12. How many years (on average) did you take part in moderate or strenuous exercise 4 or more hours per week?

- ☐ Less than 1 year
- ☐ 1 - 5 years
- ☐ 6-10 years
- ☐ 11 - 15 years
- ☐ 16-20 years
- ☐ More than 20 years

EXERCISE

13. On average, during the past year how much time per week did you spend at each of the following activities?

[illegible]

14. On average during the past year, how many hours per week did you spend:

Activity	AVERAGE HOURS PER WEEK								
	Zero hours	One hour	2-4 hours	5-10 hours	11-20 hours	21-40 hours	41-60 hours	61-90 hours	90+ hours
Standing or walking around at work?	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Standing or walking around at home?	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Sitting at work or while driving?	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Sitting at home?	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>

15. What is your usual walking pace outdoors?

- ☐ Easy, casual (less than 2 mph)
- ☐ Normal, average (2-2.9 mph)
- ☐ Brisk pace (3-3.9 mph)
- ☐ Very brisk/striding (4 mph or faster)
- ☐ Unable to walk

16. How many flights of stairs (not individual steps) do you climb daily?
(1 flight = 10 steps)

- ☐ 2 flights or less
- ☐ 3-4 flights
- ☐ 5-9 flights
- ☐ 10-14 flights
- ☐ 15 or more flights

SLEEP HISTORY

THERE IS INTEREST IN WHETHER SLEEP PATTERNS ARE RELATED TO HEALTH.
PLEASE ANSWER THE FOLLOWING QUESTIONS ABOUT YOUR SLEEP HABITS.

17. How many hours do you usually sleep at night (or your main sleep period) on weekdays or workdays?

- ☐ Less than 6 hours
- ☐ 6 hours
- ☐ 7 hours
- ☐ 8 hours
- ☐ 9 hours
- ☐ 10 hours
- ☐ More than 10

18. How many hours do you usually sleep at night (or your main sleep period) on weekends or your non-work days?

- ☐ Less than 6 hours
- ☐ 6 hours
- ☐ 7 hours
- ☐ 8 hours
- ☐ 9 hours
- ☐ 10 hours
- ☐ More than 10

19. On average, during the past year, how often have you felt sleepy during the day, no matter how much sleep you had?

- ☐ Never
- ☐ Rarely (One day per month or less)
- ☐ Sometimes (2-4 days per month)
- ☐ Often (5-15 days per month)
- ☐ Almost always (16-30 times per month)

20. On average, during the past year, how often have you ever taken sleeping pills, melatonin, or other medicine to help you sleep?

- ☐ Never
- ☐ Rarely (Once per month or less)
- ☐ Sometimes (2-4 times per month)
- ☐ Often (5-15 times per month)
- ☐ Almost always (16-30 times per month)

21. The questions below ask how many servings of the following items you ate or drank during the past year.

21. The questions below ask how many servings of the following items you ate or drank during the past year.

[illegible]

BEVERAGE HISTORY

22. In the past year, about how often did you drink tea (not herbal) (hot or iced, caffeinated or decaffeinated)?

- ☐ Never
- ☐ 1-3 cups per month
- ☐ 1-2 cups per week
- ☐ 3-4 cups per week
- ☐ 5-6 cups per week
- ☐ 1 cup per day
- ☐ 2 cups per day
- ☐ 3 cups per day
- ☐ 4 cups per day
- ☐ 5 or more cups per day

If you drink hot tea, do you add milk to your hot tea?

- ☐ No
- ☐ Yes

23. In the past year, about how often did you drink wine (1 glass = 5 oz. serving)?

- ☐ Never
- ☐ 1-3 glasses per month
- ☐ 1-2 glasses per week
- ☐ 3-4 glasses per week
- ☐ 5-6 glasses per week
- ☐ 1 glass per day
- ☐ 2 glasses per day
- ☐ 3 glasses per day
- ☐ 4 glasses per day
- ☐ 5 or more glasses per day

24. If you drink wine, what kind of wine do you usually drink?

- ☐ White
- ☐ Red
- ☐ Blush

THANK YOU!

Please check to make sure you have not accidentally skipped any pages.

Please return booklet in the postage-paid envelope to:

Johns Hopkins Research Center
P.O. Box 2067
Hagerstown, MD 21742-2067